Vol. 106, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 31, 1982 Pages 526-532

A NEW METABOLIC REACTION OF DIAZIRIDINES BY RAT LIVER MICROSOMES

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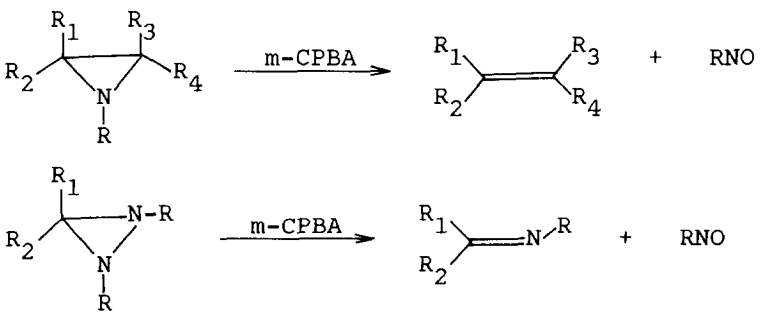
Received April 20,1982

The reaction of 1,2-dimethyl-3-p-chlorophenyldiaziridine with rat liver microsomes in vitro was studied. The products of the metabolic reaction were methylamine, p-chlorobenzaldehyde and p-chlorobenzylalcohol. The initial step of the metabolic reaction, which led to decomposition of the diaziridine ring, was surprisingly a reductive reaction.

Diaziridines are hetero three-membered ring compounds and their structure suggests that they may have chemical properties similar to those of aziridines. For example, both ring systems showed a common fragmentation reaction toward the oxidation using m-chloroperbenzoic acid (1,2).



Thus, we supposed the biological properties of diaziridines should also be similar to those of aziridines. Actually, however, the metabolic reactions of both chemical species by rat liver microsomes were surprisingly different,

0006-291X/82/100526-07$01.00/0

one was oxidative (3) and the other was reductive. Here, we want to report a new metabolic reaction of diaziridine.

MATERIALS AND METHODS

1,2-Dimethyl-3-p-substituted phenyldiaziridines (1) were prepared by the method of Schmitz et al. (4) from corresponding aldehydes.

General Procedure of Transformation of Diaziridines in Microsomal Suspension. Washed liver microsomes were prepared from normal SLC-Wistar strain male rats, weighing about 300 g, by the differential centrifugation method (5). The protein concentration of the microsomes was determined by the biuret reaction (6) using bovine serum albumin as standard.

The metabolic reactions were carried out in a complete system consisting of 50 mM Tris-HCl buffer (pH 7.4) containing 150 mM KCl, 10 mM $MgCl_2$, 1 mM nicotinamide, 1 mM pyrophosphate, 30 mg of protein of microsomes, 0.01 mM of diaziridines and an NADPH-generating system (0.9 mM NADP, 10 mM glucose-6-phosphate and 12.5 units of glucose-6-phosphate dehydrogenase) in a final volume of 5 ml. Unless otherwise stated, incubation was carried out aerobically at 37 °C for 30 min with moderate shaking. This procedure was almost the same as that described previously in the study of the aziridine derivative (3). After the reaction time was over, 2 ml of 2 N NaOH solution was added or the reaction mixture was heated in boiling water for 90 sec and then immediately cooled by immersion in ice water to stop the reaction (7). Next, as an extraction solvent and an internal reference, 1,2-dichloroethane (2 ml) and $C_{14}H_{30}$ were added, respectively. The amount of $C_{14}H_{30}$ was regulated to allow an approximately equivalent area for the reaction products in VPC. After vigorous shaking of this mixture for 5 min at room temperature and 15 min of centrifugation, a clear solution was obtained for VPC analysis which was conducted using 20% Carbowax-20M, 5% Silicon UCW-98, 5% XE-60 on Chromosorb W or Porapak QS in 4 mm x 1-3 m glass columns. The reaction products were identified mainly by GC/MS or by comparison of the retention times on three different kinds of VPC columns with those for commercially purchased authentic samples. The yields of the reaction products were calculated from the VPC peak areas.

RESULTS

In preliminary experiments, 1,2-dimethyl-3-p-substituted phenyldiaziridines 1 were treated with rat liver microsomes in the complete system. The reaction products were p-substituted benzaldehyde, benzylalcohol and methylamine. The products and percentages of recovered material are shown in Table I.

Among the diaziridines in Table I, 1-CH_3O was very unstable. It disappeared immediately from the microsomal solution and we could not detect the products. 1-CN and 1-NO_2

Table I. Percentages of Products Obtained in the Reaction of 1,2-Dimethyl-3-parasubstituted phenyldiaziridines by Rat Liver Microsomes

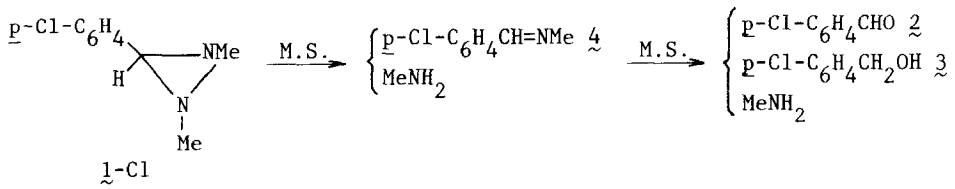
p-substituent group on 1	p-substituted benz-aldehyde (%)	p-substituted benzyl alcohol (%)	methyl-amine (%)	recovered 1 (%)
CH_3O-	-	-		-
CH_3-	8.4	6.7		56
H-	6.0	9.3		73
Cl-	5.6	13.9	quantitative[a]	70
CN-	<1	<2		57
NO_2-	-	-		52

[a] This corresponds to twofold molar of methylamine on the base of the amount consumed of the starting material. Analysis was carried out using Porapak-QS VPC column treated with 1% NaOH and 10% tetraethylenepentamine. The generation of methylamine was observed in the microsomal solution allowed to stand at room temperature for prolong period. Therefore the experiment was carried out quickly and the yield of methylamine was corrected.

also gave unsatisfactory products. 1-Cl gave the best results. In repeated studies of the reaction, it usually gave 90% or more of products 2 and 3 with some recovery of 1-Cl. Thus we used 1-Cl for our extensive study of the metabolic reaction of diaziridines in rat liver microsomes.

The results observed for several reaction conditions for 1,2-dimethyl-3-p-chlorophenyldiaziridine 1-Cl are given in Table II. The result obtained under standard conditions using a complete system and air is given as Run 5 of Table II. The metabolic reaction of diaziridine was clearly inhibited by the absence of NADPH or microsomes from the complete system as shown by Runs 8 and 9, which indicates that the reaction proceeded under the influence of hepatic enzymes in rat liver microsomes. In addition to these, the effect of carbon monoxide as shown in Run 7 of Table II suggested the reaction occurs by cytochrome P-450 catalysis (8).

Surprisingly, the metabolic reaction of diaziridine was, as described in Table II, accelerated by the replacement of



oxygen by an inert gas such as argon or nitrogen. The fastest consumption was accompanied by very good yields of 2 and 3 when pure argon was used for the gas phase. Therefore, the initial step of the reaction of diaziridine 1-Cl must have been a reductive fragmentation process of the three-membered ring (9). The reaction should have occurred on the reduced form of cytochrome P-450.

Table II. Conditions and Product Distributions Observed in the Reaction of 1,2-Dimethyl-3-p-chlorophenyldiaziridine 1-Cl by Rat Liver Microsomes (37 °C, 30 min incubation)[a]

exp. no.	system	gas phase[c]	aldehyde 2 (%)	alcohol 3 (%)	recovered 1-Cl (%)
1	Complete[b]	Argon or nitrogen	19.8	37.6	26
2	Complete	0.5% O_2 in Ar	15.2	36.5	32
3	Complete	1% O_2 in Ar	13.8	31.5	40
4	Complete	5% O_2 in Ar	10.8	27.7	50
5	Complete	21% O_2 in N_2[d]	10	18.9	63
6	Complete	Only O_2	2.7	11.5	70
7	Complete	CO	6.4	<1	95
8	Without NADPH	Air	<1	0	97
9	Without microsomes	Air	<1	<1	99

[a] After the incubation, the reaction was interrupted by the heating at 100 °C for 90 sec then analyzed by VPC according to the method described in the Experimental Section.

[b] The complete system, as described in the text, contained all components required for microsomal drug metabolism.

[c] For the modified experiment for the gas phase, a Thumberg tube was used.

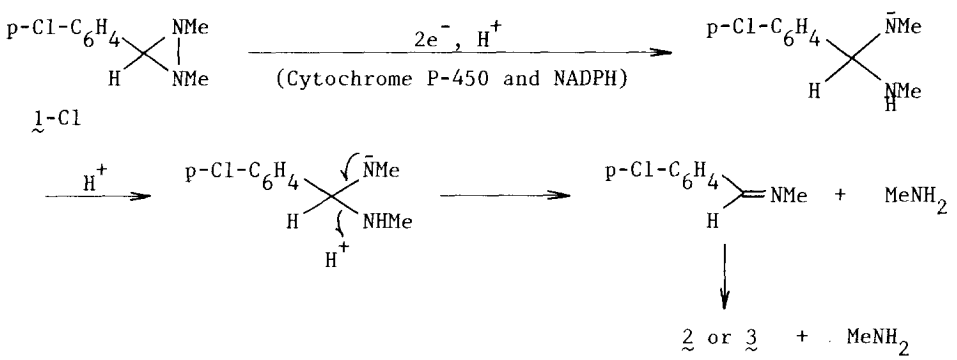
[d] This is air. Nitrogen and argon gave same results.

The yields were calculated on the basis of the amount of consumed starting material.

DISCUSSION

The active site of P-450 contains an iron protoporphyrin moiety in a large relatively open hydrophobic cleft or depression in the surface of the apoprotein. According to the scheme proposed by White and Coon (10), the oxygen-dependent metabolic reaction should be initiated by the participation of substrate at the hydrophobic site and by the activation of oxygen of the ferrous dioxygen complex formed at the active center.

In the initial stage of the metabolic reaction of diaziridines, however, the complex formation of diaziridine on the sixth coordination position of the iron probably occurs more predominantly than the adsorption onto a hydrophobic position, with which it is in equilibrium. After this, coordinated diaziridines should be decomposed by the attack of electrons and a proton onto nitrogen atom as shown below.



These possibilities are supported by literature showing that ferrous cytochrome P-450 combines readily with a number of small ligands, such as CO (11), NO (12), RCN (13), pyridine derivatives (14) and hydrazines (15).

Recently, Kato and Sugiura reported that tertiary amine N-oxides are reduced to the corresponding tertiary amines in liver microsomes by the reduced form of cytochrome P-450 (16).

The metabolism of hydrazines, which form alkylamines using intestinal microflora, was also reported by Bolton and Griffiths (17). We consider that the reductive fragmentation of diaziridines to the corresponding imine and methylamine in rat liver microsomes has a very similar reaction character and these reactions proceed via essentially similar mechanisms on the surface of cytochrome P-450.

Acknowledgment — We wish to thank Dr. T. Matsubara and Mr. A. Touchi for their helpful discussion and assistance with the experiments.

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